The Tar Fraction of Cigarette Smoke Does Not Promote Arteriosclerotic Plaque Development

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In addition to being the single greatest known environmental cause of cancer, cigarette smoke (CS) is also a major contributor to heart disease. We reported previously that 1) inhalation of either mainstream or sidestream CS promotes aortic arteriosclerotic plaque development; 2) 1,3 butadiene, a vapor-phase component of CS, promotes plaque development at 20 ppm, which at the time was only 2 times higher than the threshold limit value; and 3) individual tar fraction carcinogens in CS, including polynuclear aromatic hydrocarbons (PAHs) and nitrosamines, either do not promote plaque development or do so only at high concentrations. These results suggested that the tar fraction is not the primary source of plaque-promoting agents in CS. We asked whether repeated exposure to the tar fraction of CS, collected in a cold trap (TAR), promotes plaque development in an avian model of arteriosclerosis. Acetone extracts of mainstream CS tar from burning, unfiltered reference cigarettes were solubilized in dimethyl sulfoxide (DMSO) and injected weekly into cockerels for 16 weeks (25 mg/kg/week). Positive controls were injected weekly with the synthetic PAH carcinogen, 7,12 dimethylbenz(*a*)anthracene (DMBA) dissolved in DMSO and negative controls were injected with DMSO. Plaque location and prevalence did not differ from group to group. Morphometric analysis of plaque cross-sectional areas showed that plaque sizes, which are log-normally distributed, were significantly larger in the DMBA cockerels compared to both the TAR and DMSO groups. There were no significant differences in plaque size between DMSO and TAR cockerels. The results reported here, combined with other recent findings, support the conclusion that the primary arteriosclerotic plaque-promoting components of CS are in the vapor phase. *Key words*: arteriosclerotic plaques, cigarette smoke, cigarette tar (fraction), cockerels. *Environ Health Perspect* 104:1108–1113 (1996)

The primary causes of death in the United States are heart disease/stroke (864,000 deaths in 1990) and lung cancer (141,000 deaths in 1990) (*1*). In addition to the obvious effects on the victims and their families, the costs of these diseases are very high in terms of lowered productivity, increased costs, and added stress to the health care system. The very strong association of smoking with these diseases is particularly striking. Nearly 90% of all lung cancers are attributible to cigarette smoking, and smoking is also associated with cancers at a number of other sites, including the mouth, larynx, pancreas, and bladder (*2*). The incidence of coronary heart disease is twice as high among smokers as nonsmokers and four times higher for heavy smokers as nonsmokers. In addition, death rates from coronary heart disease are 70% higher for smokers and more than twice as high for heavy smokers than for nonsmokers (*3*).

The deleterious health effects associated with smoking arise from two main sources—mainstream smoke and environmental tobacco smoke (ETS; second-hand smoke; passive smoke). Mainstream smoke, which emerges from the mouth end of a cigarette, is the primary source of harmful agents to smokers. Eighty-five to 90% of ETS comes from aged and diluted sidestream smoke (from the burning end of a cigarette), with the remainder coming from exhaled mainstream smoke. In recent years there has been a great increase in public sensitivity to the perceived, potentially harmful effects of ETS exposure on nonsmokers. Epidemiological studies have lent support to these perceptions. The U.S. Environmental Protection Agency (EPA) has classified sidestream smoke as a human carcinogen and has attributed ~3,000 excess lung cancer deaths in the United States each year to involuntary exposure to ETS (*4*). A role for ETS in the appearance of precancerous lung lesions has also been reported (*5*). The effects of ETS on cardiovascular disease are estimated to be far more extensive. The American Heart Association has recommended that ETS be classified as a major environmental toxin and has classified ETS as a major preventable cause of heart disease (*6*). In a recent review (*7*), it was estimated that >60,000 excess preventable heart disease deaths occur yearly in the United States due to involuntary exposure to ETS. Two recent studies from our laboratory provided direct experimental support for this contention. Results from studies using the cockerel model of arteriosclerotic plaque development (*8,9*) revealed that inhalation exposure to ETS at levels equal to or even below those that can be encountered by people in smoke-filled environments is sufficient to promote arteriosclerosis (*10,11*). Atherosclerosis studies in cholesterol-fed, smoke-exposed rabbits confirm these findings (*12*).

Many of the same chemicals are present in mainstream smoke and ETS, although their relative abundances in the two smoke fractions are different (*13*). Despite the overwhelming evidence implicating cigarette smoke (CS) in the development of both cardiovascular disease and lung cancer, it has been virtually impossible to identify a single component of CS that, at environmentally relevant levels, is responsible for any of the observed health effects. The major reason is that although more than 4,500 compounds, many of which can be cytotoxic or carcinogenic, have been identified in CS (*13*), most are present in concentrations deemed to be too low to be effective individually. Polynuclear aromatic hydrocarbon (PAH) carcinogens, e.g., benzo(*a*)pyrene [B(*a*)P], and nitrosamines in the tar fraction are considered primarily responsible for the effects of CS on lung cancer and heart disease. Indeed, these and related compounds are effective at inducing or promoting these diseases experimentally, but only when administered at levels many times (often orders of magnitude) higher than the levels at which they are found in mainstream smoke or in ETS (*14–22*).

Recently, we reported results of a study in which we tested the effects of two CS components on arteriosclerotic plaque development in cockerels (*23*). The tar fraction nitrosamine 4-(*n*-methyl-*n*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which is carcinogenic at high concentrations in rodents (*14,18*), had no effect on plaque development in cockerels when injected biweekly at doses of 10 mg/kg. The injection protocol we followed is effective at demonstrating the plaque-promoting effects of a variety of chemicals and environmental agents (*16,19*). However, inhalation expo-

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sures to the volatile alkene 1,3 butadiene, a vapor phase component of both mainstream smoke and ETS, effectively promoted plaque development at doses (20 ppm) only 2 times higher than the threshold limit value (TLV) at the time of the experiments. (The 8-hr time-weighted average TLV for 1,3 butadiene has recently been reduced from 10 ppm to 2 ppm). These results suggest that the plaque-promoting components of CS reside mainly in the vapor phase. To test this suggestion, we carried out the plaque-promotion experiments described here. We injected either solubilized concentrated tar or a PAH carcinogen, 7,12 dimethylbenz(*a*)anthracene (DMBA), into cockerels. As expected, based on previous plaque development studies with carcinogens (*15,16,19,24*), DMBA was a strong promoter of plaque development; however, the solubilized CS tar was totally ineffective. These findings support the conclusion that the major arteriosclerotic plaque-promoting components of CS reside in the vapor phase rather than in the tar fraction.

Materials and Methods

Two- to 3-week-old white leghorn chicks (Hyline International, Dallas Center, IA) were quarantined for 2 weeks, during which time they were acclimated to a 12 hr light/dark cycle and observed for anomalous behavior and disease. As in our previous studies, only males were used. Cockerels were distributed randomly into three groups of 10 each and were housed in stainless steel dog cages (2–4/cage). Care and treatment were in accordance with established guidelines. Cockerels received food (Chick Starter Grower, Purina, St. Louis, MO) and water *ad libitum*. They were weighed weekly and their health was monitored daily. Exposures began at 5 weeks of age.

Tar was extracted in a well-ventilated hood from 20 2R1 unfiltered reference cigarettes at a time (Tobacco and Health Research Institute, University of Kentucky, Lexington, KY). An all-glass smoking machine was used; this machine consisted of a cylinder in which the tobacco was burned connected to a series of eight tubes cooled to -40° C by an alcohol-dry ice bath (*25*). The tubes were connected with ground glass joints. Each tube was filled with 20 ml of acetone. A 75 cc volume of air was drawn through the smoking system (11times per minute) by a Palmer respiration pump (Fisher, Valley Forge, PA). The temperature of the burning tobacco ranged from 500°–700° F. The crude tar fractions collected from each of the tubes were pooled and stirred overnight at room temperature. The crude tar was resuspended in acetone, aliquoted, and frozen until use. The acetone was evaporated before the tar was solubilized in dimethylsulfoxide (DMSO, Fisher, Valley Forge, PA). The extraction process yielded ~850 mg of tar per pack. The tar was prepared fresh monthly. (The tar tested here is more rigorously classified as cigarette smoke condensate, since it is collected in a cold trap; however, for purposes of simplicity we will refer to it as TAR). Cockerels were injected weekly (intramuscularly; im) according to our standard protocols (*16,19*). The tar dose was 25 mg/kg/week and the DMBA dose (Sigma, St. Louis, MO) was 10 mg/kg/week. Tar and DMBA were solubilized in DMSO. Control cockerels were injected with 1 ml DMSO/kg/week. All animals were sacrificed humanely at 21 weeks of age after receiving 16 weekly injections each. Aortas were opened longitudinally, rinsed with saline, and fixed in 10% buffered formalin. Aortas were sectioned transversely at 5–6 mm intervals from the ischiatic bifurcation to the thoracic aorta (nine sections per cockerel). Following paraffin embedding, 5 μm thick sections were cut from the distal face of each block. Sections were exposed to the Verhoff-van Gieson stain (*26*) to distinguish plaque from underlying artery wall and to stain collagen (red) and elastin (black). The cross-sectional areas of plaques present in each plaque-containing segment were measured microscopically, as described previously (*16,19*), i.e., maximal depth × width of plaques. All segments with visible plaque were scored. When a consecutive group of plaque-containing segments within an aorta was broken by a segment with no visible plaque, the aorta was scored as having two plaques. All samples were coded prior to analysis.

Statistical Analysis

The number of plaques per cockerel and the numbers of plaque-containing segments per cockerel in the three groups were each compared by ANOVA. Plaque sizes, which were log-normally distributed, were log-transformed for statistical evaluation. The dependence of plaque size both on location along the aorta and on treatment was determined by a 2-factor ANOVA using the SuperANOVA program (Abacus Concepts, Berkeley, CA). Least squares analysis was used to compare plaque size for any segment among the three groups.

Results

There were 10 cockerels in each group at the start of the experiment. One cockerel in the DMSO group died in the fourteenth week of the study and was not included in the data analysis. There were no significant differences in body weights between cockerels in any of the groups at any time point (data not shown). All major organs appeared grossly normal at autopsy.

Table 1. Effect of treatments with DMBA, cigarette tar (TAR), or DMSO on plaque prevalence in cockerel aortas

	No. cockerels	Plaques/ cockerel[a]	Plaque segments/ cockerel[a]
DMSO	9	1.44 ± 0.17	8.11 ± 0.23
DMBA	10	1.30 ± 0.15	7.40 ± 0.35
TAR	10	1.70 ± 0.20	7.40 ± 0.21

[a]All values are mean ± SEM.

There was no significant effect of treatment on the number of plaques per cockerel (1.3–1.7) or on the number of aortic segments (7.4–8.1) with measurable plaque per cockerel (Table 1). There were 73 segments with plaque in the DMSO group and 74 each in the DMBA and TAR groups. As reported previously (*9–11,16,19*) plaque sizes were log-normally distributed, i.e., mean plaque sizes exceeded median values in all three groups (data not shown).

Both treatment and location were associated with a significant increase in plaque size within each group of cockerels. Figure 1 displays the association of location with plaque size for abdominal aorta segments from all the cockerels in each of the three groups. The largest plaque sizes are found in the three most distal segments of the abdominal aorta, regardless of treatment. The effect of treatment on plaque size is shown in Figure 2. As expected, DMBA treatment resulted in a significant increase in plaque size compared to both TAR and DMSO ($F = 4.84$; $p = 0.009$). In contrast, TAR treatments had no significant effect on plaque size. In fact, the effects of TAR and DMSO were indistinguishable. When the effects of treatment and location were combined, the aortic segments with the largest plaques were segments 1 and 3 in the DMBA group. Plaque sizes in these segments were significantly larger than in the same segments in the TAR ($p = 0.05$; 0.026) and DMSO ($p = 0.002$; 0.015) groups (Table 2). When DMSO and TAR groups were compared, there were no significant differences in plaque size for any aortic segment. Thus, weekly injections of concentrated cigarette tar into cockerels, according to a protocol that has been used successfully to identify arteriosclerotic plaque-promoting agents in the environment, were without effect on plaque promotion.

Discussion

The data supporting a role for cigarette smoking in lung cancer (and other cancers) and in heart disease are overwhelming. The

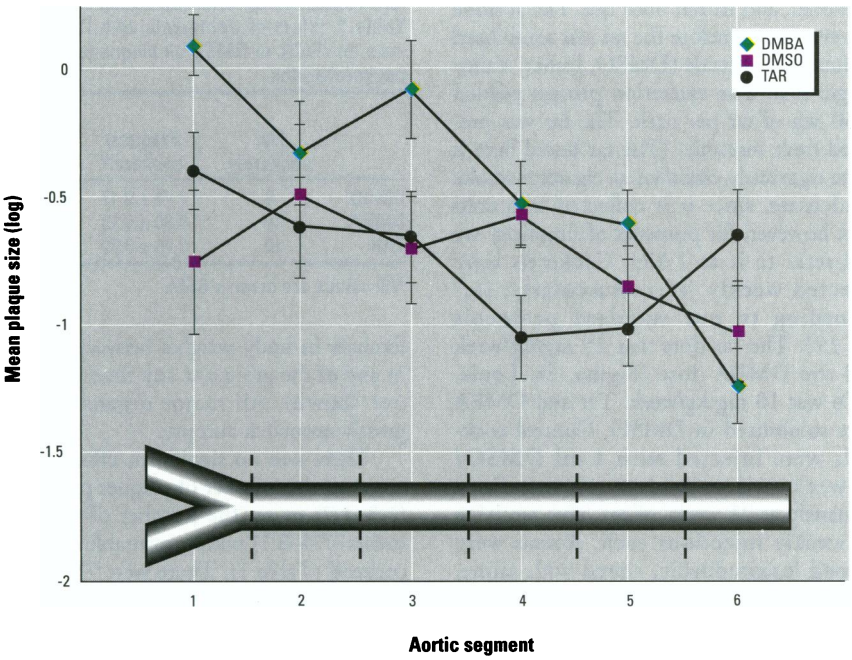

Figure 1. Relationship between size and location of arteriosclerotic plaques in the abdominal aortas of cockerels. The means of the log-normally distributed plaque sizes are shown for the six most distal contiguous segments of the abdominal aorta. For DMSO, $n = 9$, and for DMBA and TAR, $n = 10$. The results show that treatment did not alter plaque location and that the largest plaque sizes were found in the most distal segments of the aorta, regardless of treatment.

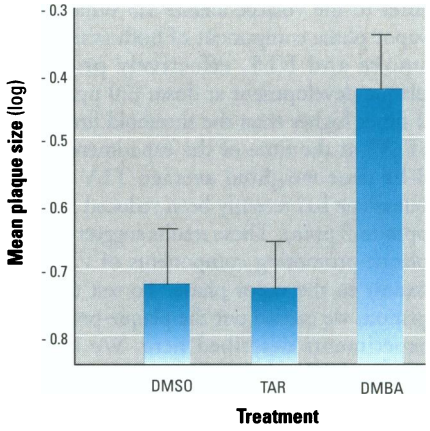

Figure 2. Effect of repeated injections of cigarette tar on development of arteriosclerosis. The means of the log-normally distributed plaque sizes are shown for cockerels injected weekly with cigarette tar (TAR), DMBA (positive control), or DMSO (negative control). The values represent average total plaque size for the six most distal aortic segments from cockerels in each group. For DMBA and TAR, $n = 60$, and for DMSO, $n = 54$. $df = 2$; $F = 4.837$; $p = 0.009$.

Table 2. Comparison of the effect of treatment with DMBA, cigarette tar (TAR), or DMSO on plaque size at aortic segments 1 and 3 (see Fig. 1)

	t-value	*p*-value
Aortic segment #1		
(DMBA vs. DMSO)	3.20	0.002
(DMBA vs. TAR)	1.97	0.050
(DMSO vs. TAR)	-1.35	0.180
Aortic segment #3		
(DMBA vs. DMSO)	2.45	0.015
(DMBA vs. TAR)	2.25	0.026
(DMSO vs. TAR)	-0.19	0.851

association of smoking with lung cancer was clearly established over 40 years ago (*27–29*), and the association of smoking with heart disease was recognized by the U.S. Surgeon General over 30 years ago (*30*). It is widely assumed that tar fraction components of cigarette smoke—especially the PAH carcinogens (e.g., B(*a*)P) and nitrosamines (e.g., NNK)—are primarily responsible for these profound health effects (*14,17,18,20–22,31–37*). The results presented here combined with others we have recently published (*23*) demonstrate that, at least as far as heart disease is concerned, this assumption should be reexamined. The results here show clearly that repeated injections of concentrated solutions of tar from burning unfiltered cigarettes have no effect on the development of arteriosclerosis in a sensitive model of the disease. Cockerels are sensitive to plaque-promoting effects of certain environmental contaminants whether they are administered by injection (*15,16,19,23*), as was the case here, or by inhalation (*10,11,23,38*).

There are two characteristics of this animal model of arteriosclerosis that make it particularly valuable for studies of the type described here. The first is that chickens develop naturally occuring fibromuscular arteriosclerotic plaques that appear spontaneously in the abdominal aorta (*8*). In the absence of exogenous stimuli, these spontaneous plaques remain microscopic for most of the first year of life (*9,24*). They are probably a form of the intimal cell masses or intimal pads that have been described as sites of focal intimal proliferation in both young humans and young animals (*39–42*). The second characteristic is that both mainstream (*38*) and sidestream (*10,11*) CS, as well as some carcinogens present in CS (*15,16,19,24*), stimulate development of these spontaneous plaques, whereas other tobacco components including the tobacco-specific nitrosamine NNK (*23*) and carbon monoxide (*43*) have no detectable effect on plaque development *in vivo*. The results presented here confirm and extend observations we made previously that, in relation to plaque development, the active environmental agents function as promoters (stimulating development of pre-existing lesions via repetitive treatment) rather than as initiators (causing *de novo* appearance of lesions following a single treatment (*10,11,15,16,19, 23,38*). The positive control here, the PAH carcinogen DMBA, which is often used as an initiator in carcinogenesis studies, did not elicit appearance of new plaques. Rather, as in our previous studies (*15,16,19,24*) it accelerated development of preexisting plaques in the most distal portions of the aorta. The test agent, tar extracted from CS, containing numerous carcinogens (albeit at relatively low concentrations), was no more effective than DMSO, the solvent control, at stimulating development of aortic plaques. Although this distinction between plaque promoters and initiators is more functional than mechanistic, a recent mechanistic study of the effect of DMBA on tumor development in mouse epidermis also emphasized the primary role of DMBA as a promoter rather than as an initiator of tumorigenesis (*44*).

The unfiltered 2R1 cigarettes used both in this study and in an earlier mainstream smoke study (*38*) and the filtered 1R4F cigarettes used in our recent sidestream smoke studies (*10,11*) are standardized research cigarettes produced by the Tobacco and Health Research Institute at the University of Kentucky. Mainstream smoke tar levels from the 2R1 cigarettes (33 mg/cigarette) are ~3.5 times higher than those from 1R4F cigarettes (9 mg/cigarette). In the earlier 2R1 mainstream smoke study (*38*), cockerels inhaled mainstream smoke 2 hr/day for 16 weeks, resulting in a moderate but statistically significant increase in plaque size. Sidestream smoke from 1R4F cigarettes has ~10%

more tar than does the mainstream smoke (*13*). The 1R4F sidestream smoke exposures were 3 times longer (6 hr/day) than were the earlier 2R1 mainstream smoke exposures. Thus, the dose × time product for tar was similar in the two sets of exposures. However, the results were not equivalent. Inhalation of sidestream smoke accelerated aortic plaque development far more than did inhalation of mainstream smoke.

In the experiments reported here to test whether the tar fraction of CS promotes plaque development, the cockerels were exposed to environmentally relevant levels of CS tar fraction. When a 2R1 unfiltered cigarette is smoked under standard conditions (i.e., those specified by the Federal Trade Commission)—a 35 ml puff of 2 sec duration, one time per minute, down to a butt length of 23 mm—the dose to a typical smoker has been calculated to be 33 mg of tar. For a one pack per day smoker weighing 70 kg, the weekly tar dose is 66 mg/kg (33 mg/cigarette × 20 cigarettes × 7 days/70 kg). This compares favorably with the concentrated tar dose administered here—25 mg/kg/week.

The difficulties in demonstrating directly that specific smoke components are responsible for the respiratory and cardiovascular health effects of smoking are due in large part to 1) the extraordinary complexity of tobacco smoke; 2) the relatively low concentration of most of the individual components in the smoke; and 3) the different relative concentrations of chemicals in mainstream versus sidestream smoke. For example, B(*a*)P levels are ~16 times higher in sidestream than in mainstream smoke of 1R4F cigarettes, 148 ng versus 9 ng (*13*). However, to provide B(*a*)P via inhalation of sidestream smoke at a level equivalent to the injected B(*a*)P level used in our earlier plaque acceleration study, i.e., 40 mg/kg/week(*19*), requires the equivalent of nearly 300,000 cigarettes per week per kg cockerel.

The lower health risks associated with sidestream versus mainstream smoke are probably explained by the fact that sidestream smoke components diffuse through the air while mainstream smoke components are delivered directly to the mouth of an active smoker. Therefore, the latter inhales greater amounts of smoke components than does a passive smoker. Nevertheless, the health effects arising from exposure to ETS, largely discounted until recently, may be considerable. After determining ETS exposures in human populations, assessing lung cancers in humans and animals exposed to ETS, and evaluating the association between ETS and lung cancer according to its previously established criteria for causality, the EPA declared that the highly complex mixture called ETS is itself a class A human carcinogen (*4*). Although the estimate of the number of excess lung cancers due to ETS exposures are relatively low, ~3,000/year in the United States (*4*), the estimated number of excess heart disease deaths due to ETS exposure is more than an order of magnitude higher (*7,45,46*). Studies from at least two laboratories, each using different animal models, exposure protocols, diets, and cigarettes as the ETS source, have shown clearly that ETS inhalation causes significant augmentation of arteriosclerotic plaque development (*10–12*).

With the exception of carbon monoxide, a vapor phase component that plays no direct role in arteriosclerosis in cockerels (*43*) and only a small role at best in exacerbating other manifestations of heart disease in humans (*47*), investigations of the cancer- and arteriosclerosis-associated effects of CS have long focused on the tar fraction and its components (*25,48,49*). Evidence for activation of cytochrome P450 enzymes, which is necessary for the metabolism of the PAH carcinogens, has been reported in the vascular system following *in vivo* and *in vitro* exposures to carcinogens that are present in cigarette tar (*22,31,32,50*). However, the concentrations of individual PAH carcinogens, e.g., B(*a*)P or 3-methylcholanthrene, needed to activate P450 enzymes in vascular smooth muscle or endothelial cells are orders of magnitude higher (*17,22,31*) than the concentrations of these and related compounds in cigarette smoke (*13*). Thus, there is little direct evidence of a role for environmentally relevant levels of tar fraction compounds in the development of arteriosclerosisis.

Both the experiments described and the literature reviewed here focus on arteriosclerotic plaques and not on lung tumors; however, the experimental carcinogenesis literature also raises the question of whether environmentally relevant levels of tar fraction components are primarily responsible for CS-associated carcinogenesis. A mid-1960s review and summary of cigarette tar and carcinogenesis studies dating back to the early 1900s showed cigarette tar to be largely ineffective as a whole carcinogen. In addition, the tar was both a very weak initiator and, at best, only a fair promoter of carcinogenesis (*33*).

Even more than B(*a*)P, NNK is regarded as one of the most potent carcinogens in CS (*20,21*); however, the potency of NNK as an experimental lung carcinogen is associated with extended exposures, repeated doses of much higher levels than are found in cigarettes, or both (*14,20,35,51*). In rodent carcinogenesis studies, multiple NNK doses equivalent to 100–1000 mg/kg or more have been reported (*14,18,35*). A dose of 3 mg of NNK (~15 µmol) is equivalent to the total NNK in the sidestream smoke from 7,500 1R4F filtered reference cigarettes or in the mainstream smoke from 37,500 of the same cigarettes (*13*). Evidence questioning the role that these prominent tar fraction carcinogens play in experimental lung tumor development also has been provided recently by analysis of Ki-*ras* mutation spectra. NNK and PAHs elicit codon 12, but not codon 61, mutations in Ki-*ras*, (*36,37*). However, mice exposed to sidestream smoke for 6 months exhibited primarily codon 61 Ki-*ras* mutations in lung tumors (*52*).

Finally, another set of recent findings provides evidence that vapor phase components of CS may represent a greater health risk than do tar fraction components (Bombick et al., unpublished data). These authors tested *in vitro* cytotoxicity of tar condensate and filtered smoke, both from conventional cigarettes and from experimental cigarettes with a charcoal-impregnated filter that selectively removes vapor phase components from the smoke. The tar fractions from the two types of cigarettes did not differ in cytotoxicity; however, the smoke passing through the experimental filter was 50% less cytotoxic than smoke passing through the conventional filters.

The results described here represent the first clear attempt to affect the course of arteriosclerotic plaque development *in vivo* by direct administration of the tar fraction of CS. No effect on plaque development was detected.

This result, combined with recent findings from our laboratory and others, indicates that, at least as far as arteriosclerotic plaque development is concerned, the CS components responsible reside in the vapor phase, not in the tar fraction.

Summary of the effect of cigarette smoke (CS) and its components on development of arteriosclerotic plaque

Experimental data show that inhalation of either mainstream or sidestream CS at environmentally relevant levels results in accelerated arteriosclerotic plaque development in cockerels (*10,11,38*). Inhalation of sidestream CS combined with a high cholesterol diet also accelerates atherosclerosis in rabbits (*12*). Epidemiological data show that life expectancy for ex-smokers approaches that for nonsmokers as the time since smoking cessation increases (*3*). Thus, CS acts as a promoter of cardiovascular disease.

The tar fraction of cigarette smoke contains many carcinogens, including both

PAH carcinogens and *N*-nitrosamines, at very low concentrations (*13*).

When the PAH carcinogens are delivered to artery wall cells at high concentrations, the carcinogens can be activated metabolically by cytochrome P450 enzymes in the artery wall (*17,22,31,32,48*).

If PAH carcinogens are injected into cockerels at concentrations orders of magnitude higher than are found in the environment, accelerated plaque development results (*15,16,19,24*). The most prominent *N*-nitrosamine in CS, NNK, has no effect on plaque development after repeated administration at a moderately high concentration (*23*).

Inhalation of environmentally relevant levels of 1,3 butadiene, a CS vapor phase component, accelerates plaque development in cockerels (*23*).

Injection of concentrated cigarette tar has no effect on plaque development in cockerels.

Thus, we conclude that the arteriosclerotic plaque-promoting components of cigarette smoke reside primarily in the vapor phase.

References

1. Prevention 93–94. Atlanta, GA:Centers for Disease Control and Prevention, 1995.
2. Cancer Facts and Figures, 1994. Atlanta, GA:American Cancer Society, 1994.
3. U.S. Department of Health and Human Services. The health consequences of smoking. Cardiovascular disease. A Report of the Surgeon General. Rockville, MD:Public Health Service, Office of Smoking and Health, 1982.
4. U.S. Environmental Protection Agency. Respiratory health effects of passive smoking: lung cancer and other disorders. Washington: Office of Health and Environmental Assessment, Office of Research and Development, 1992.
5. Trichopoulos D, Mollo F, Tomatis L, Agapitos E, Delsedime L, Zavitsanos X, Kalandidi A, Katsouyanni K, Riboli E, Saracci R. Active and passive smoking and pathological indicators of lung cancer in an autopsy study. JAMA 268:1697–1701 (1992).
6. Taylor AE, Johnson DC, Kazemi H. Environmental tobacco smoke and cardiovascular disease. Circulation 86:699–702 (1992).
7. Wells AJ. An estimate of adult mortality from passive smoking. Environ Int 14:249–265 (1988).
8. Moss N, Benditt EP. The ultrastructure of spontaneous and experimentally induced arterial lesions. II. The spontaneous plaque in the chicken. Lab Invest 23:231–245 (1970).
9. Penn A, Batastini GB, Albert RE. Age-dependent changes in prevalence, size and proliferation of arterial lesions in the cockerel. I. Spontaneous lesions. Artery 7:448–462 (1980).
10. Penn A, Snyder CA. Inhalation of sidestream cigarette smoke accelerates development of arteriosclerotic plaques. Circulation 88:1820–1825 (1993).
11. Penn A, Chen LC, Snyder CA. Inhalation of steady-state sidestream smoke from one cigarette promotes arteriosclerotic plaque development. Circulation 90:1363–1367 (1994).
12. Zhu BQ, Sun YP, Sievers RE, Isenberg WM, Glantz SA, Parmley WW. Passive smoking increases atherosclerosis in cholesterol-fed rabbits. J Am Coll Cardiol 21:225–232 (1993).
13. New cigarette prototypes that heat instead of burn tobacco. Winston-Salem, NC:RJ Reynolds Tobacco Co, 1988.
14. Hecht SS, Chen CB, Ohmori T, Hoffmann D. Comparitive carcinogenicity in F344 rats of the tobacco-specific nitrosamines, n'nitrosonornicotine and 4-(*n*-methyl-*n*-nitrosamino)-1-(3-pyridyl)-1-butanone. Cancer Res 40:298–302 (1980).
15. Penn A, Batastini GB, Albert RE. Age-dependent changes in prevalence, size and proliferation of arterial lesions in the cockerel. II. Carcinogen-associated lesions. Artery 9:382–393 (1981).
16. Penn A, Batastini G, Solomon J, Burns F, Albert, RE. Dose-dependent size increases of aortic lesions following chronic exposure to 7,12 dimethylbenz(*a*)anthracene. Cancer Res 41:588–592 (1981).
17. Majesky M, Yang H-Y, Benditt EP, Juchau M. Carcinogenesis and atherogenesis: differences in monooxygenase inducibility and bioactivation of benzo(*a*)pyrene in aortic and hepatic tissues of atherosclerosis susceptible versus resistant pigeons. Carcinogenesis 4:647–652 (1983).
18. Belinsky S, Walker V, Maronpot R, Swenberg J, Anderson M. Molecular dosimetry of DNA adduct formation and cell toxicity in rat nasal mucosa following exposure to the tobacco specific nitrosamine 4-(*n*-methyl-*n*-nitrosamino)-1-(3-pyridyl)-1-butanone and their relationship to induction of neoplasia. Cancer Res 47: 6058–6065 (1987).
19. Penn A, Snyder CA. Arteriosclerotic plaque development is 'promoted' by polynuclear aromatic hydrocarbons. Carcinogenesis 9:2185–2189 (1988).
20. Hecht S, Hoffmann D. Tobacco-specific nitrosamines, an important group of carcinogens in tobacco smoke. Carcinogenesis 9:875–884 (1988).
21. Klein-Szanto A, Iizasa T, Momiki S, Garcia-Palazzo I, Caamano J, Metcalf R, Welsh J, Harris C. A tobacco-specific *N*-nitrosamine or cigarette smoke condensate causes neoplastic transformation of xenotransplanted human bronchial epithelial cells. Proc Natl Acad Sci USA 89:6693–6697 (1992).
22. Thirman M, Albrecht J, Krueger M, Erickson R, Cherwitz D, Park S, Gelboin H, Holtzman J. Induction of cytochrome CYP1A1 and formation of toxic metabolites of benzo(*a*)pyrene by rat aorta: a possible role in atherogenesis. Proc Natl Acad Sci USA 91:5397–5401 (1994).
23. Penn A, Snyder CA. 1,3 Butadiene, a vapor phase component of environmental tobacco smoke accelerates arteriosclerotic plaque development. Circulation 93:552–557 (1996).
24. Batastini G, Penn A. An ultrastructural comparison of carcinogen-associated and spontaneous aortic lesions. Amer J Pathol 114:304–309 (1984).
25. Gellhorn A. The cocarcinogenic activity of cigarette tobacco tar. Cancer Res 18:510–517 (1958).
26. Humason GL. Verhoeff elastic stain. In: Animal tissue techniques. 3rd ed. San Francisco, CA:WH Freeman Co, 1972;187–188.
27. Wynder E, Graham E. Tobacco smoking as a possible etiologic factor in bronchiogenic carcinoma. A study of six hundred eighty four proved cases. JAMA 144:329–336 (1950).
28. Wynder E, Cornfield J. Cancer of the lung in physicians. N Engl J Med 248:441–444 (1953).
29. Wynder E, Graham E, Croninger A. Experimental production of carcinoma with cigarette tar. Cancer Res 13:855–864 (1953).
30. U.S. Public Health Service. The health consequences of smoking: a report of the U.S. Surgeon General. Rockville, MD:U.S. Public Health Service, 1963.
31. Bond J, Kocan R, Benditt EP, Juchau M. Metabolism of benzo[*a*]pyrene and 7,12-dimethylbenz(*a*)anthracene in cultured human fetal aortic smooth muscle cells. Life Sci 25:425–430 (1979).
32. Bond J, Kocan R, Benditt EP, Juchau M. Oxidative and non-oxidative metabolism of polycyclic aromatic hydrocarbons in rabbit and chicken aortas and in human fetal aortic smooth muscle cells. In: Polycyclic aromatic hydrocarbons: chemistry and biological effects (Bjorseth A, Dennis A, eds). Columbus, OH:Battelle Press;1980;489–503.
33. Wynder E, Hoffmann D. Tobacco and tobacco smoke. New York:Academic Press, 1967.
34. Van Duuren B, Goldschmidt B. Cocarcinogenic and tumor-promoting agents in tobacco carcinogenesis. J Natl Cancer Inst 56:1237–1242 (1976).
35. Rivenson A, Hoffmann D, Prokopczyk B, Amin S, Hecht S. Induction of lung and exocrine pancreas tumors in F344 rats by tobacco-specific and Areca-derived *N*-nitrosamines. Cancer Res 48:6912–6917 (1988).
36. Belinsky S, Devereux T, Maronpot R, Stoner G, Anderson M. Relationship between the formation of promutagenic adducts and the activation of the Ki-*ras* protooncogene in lung tumors from A/J mice treated with nitrosamines. Cancer Res 49:5305–5311 (1989).
37. Ronai Z, Gradia S, Peterson L, Hecht S. G to A transitions and G to T transversions in codon 12 of the Ki-*ras* oncogene isolated from mouse lung tumors induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and related DNA methylating and pyridyloxobutylating agents. Carcinogenesis 14:2419–2422 (1993).
38. Penn A, Butler J, Snyder CA, Albert RE. Cigarette smoke and carbon monoxide do not have equivalent effects upon development of arteriosclerotic lesions. Artery 12:117–131 (1983).
39. Stebhins WE. Focal intimal proliferation in the cerebral arteries. Am J Pathol 36:289–295 (1963).
40. Stebhins WE. Intimal proliferation and spontaneous lipid deposition in the cerebral arteries of sheep and steers. J Atheroscler Res 5:556–568 (1965).
41. Santerre RF, Wight TN, Smith SC, Brannigan D. Spontaneous atherosclerosis in pigeons. Am J Pathol 61:1–14 (1972).
42. Scott RF, Thomas WA, Lee WM, Reiner JM, Florentin RA. Distribution of intimal smooth muscle cell masses and their relationship to early atherosclerosis in the abdominal aortas of young swine. Atherosclerosis 34:291–301 (1979).
43. Penn A, Currie J, Snyder CA. Inhalation of carbon monoxide does not accelerate arteriosclerosis in cockerels. Eur J Pharmacol 228:155–164 (1992).

44. Frenkel K, Wei L, Wei H, Karkoszka J. Polycyclic aromatic hydrocarbons (PAHs) induce oxidative stress and oxidative DNA modifications characteristic of tumor promotion. In: Polyaromatic compounds (Cavalieri E, Rogan EG, eds) Switzerland:Gordon and Breach Science Publishers, 1994;151–160.
45. Glantz SA, Parmley WW. Passive smoking and heart disease: epidemiology, physiology, and biochemistry. Circulation 83:1–12 (1991).
46. Steenland K. Passive smoking and the risk of heart disease. JAMA 267:94–99 (1992).
47. Allred E, Bleecker E, Chaitman B, Dahms T. Gottleib S, Hackney J, Hayes D, Pagano M, Selvester R, Walden S. Short-term effects of carbon monoxide exposure on the exercise performance of subjects with coronary artery disease. N Engl J Med 321:1426–1430 (1989).
48. Wynder EL, Graham E, Croninger A. Experimental production of carcinoma with cigarette tar. Cancer Res 13:855–864 (1953).
49. Orris L, Van Duuren B, Kosak A, Nelson N, Schmitt F. The carcinogenicity for mouse skin and the aromatic hydrocarbon content of cigarette-smoke condensates. J Natl Cancer Inst 21:557–561 (1958).
50. Bond J, Omiecinski C, Juchau M. Kinetics, action and induction of aortic mono-oxygenases-biotransformation of benzo(*a*)pyrene. Biochem Pharm 28:305–311 (1979).
51. Hecht S, Trushin N, Castonguay A, Rivenson A. Comparative tumorigenicity and DNA methylation in F344 rats by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and *N*-nitrosodimethylamine. Cancer Res 46:498–502 (1986).
52. Witschi H, Oreffo V, Pinkerton K. Six-month exposure of strain A/J mice to cigarette sidestream smoke: cell kinetics and lung tumor data. Fundam Appl Toxicol 26:32–40 (1995).